Potential and Challenges for Mid-Infrared Sensors in Breath Diagnostics

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***Abstract*—Exhaled breath contains more than 1000 constituents at trace level concentrations, with a wide variety of these compounds potentially serving as biomarkers for specific diseases, physiologic status, or therapeutic progress. Some of the compounds in exhaled breath (EB) are well studied, and their relationship with disease pathologies is well established. However, molecularly specific analysis of such biomarkers in EB at clinically relevant levels remains an analytical and practical challenge due to the low levels of such biomarkers frequently below the ppb (v/v) range in EB. In this contribution, mid-infrared (MIR) spectroscopic sensing techniques are reviewed for potential application in breath diagnostics. While the spectral regime from 3–20 μm has already been utilized for fundamental studies on breath analysis, significant further improvements are in demand for substantiating MIR spectroscopy and sensing techniques as a suitable candidate for clinically deployable breath analyzers. Several advantageous features including inherent molecular selectivity, real-time monitoring capability, comparable ease of operation, potentially low costs, and a compact device footprint promise reliable optical diagnostics in the MIR. Hence, while the application of MIR spectroscopy and sensing systems to breath analysis yet appear in their infancy, recent progress on advanced MIR light sources, waveguides, and device concepts forecasts next-generation optical sensing platforms suitable for addressing the challenges of *in situ* breath diagnostics.**

***Index Terms*—Absorption spectroscopy, breast cancer, chemical sensor, exhaled breath (EB), exhaled breath condensate (EBC), exposure monitoring, hollow waveguide, infrared sensor, mid-infrared (MIR), optical sensor, planar waveguide, quantum cascade laser.**

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I. Introduction

IT is well established that exhaled breath (EB) from patients suffering specific diseases contains distinctive scents, as documented for a long time in both eastern and western culture. The analysis of breath is among the oldest diagnostic techniques in medical practice characterizing diseases by specific odors [1]. For ages it was believed that expired breath from patients may contain chemicals related to specific diseases without validation of this hypothesis. In 1971, Linus Pauling pioneered the analytical assessment of breath components by gas chromatographic (GC) analysis of exhaled air, and identified a wide range of bloodborne volatile organic compounds (VOCs) exhaled after passing the blood/air interface within the lungs [2].

Also to the benefit of disease diagnostics, the scientific and technological achievements during the 20th century have brought numerous analytical diagnostic instruments to maturity and have facilitated their implementation in the medical sciences. Advanced diagnostic instruments such as electrocardiography (ECG), 3-D functional tomography (CT), and similar technologies provide more accurate vital information to medical doctors for precise diagnostic judgments on a patient's condition. Modern biomedical analytical instruments have changed and matured with the medical sciences from empirical to analytical strategies, and have assisted in broadening our understanding on physiological processes from the molecular level to cells, cell ensembles, and entire living organisms. Consequently, it is of paramount importance to monitor physiological processes associated with the human body for the diagnosis of diseases or for assessing the health status. Unique panels of molecular constituents may accompany certain physiological processes or disease pathologies as mediators, markers, or metabolic by- or end-products, etc. Hence, characteristic biomarkers addressable with appropriate analytical techniques have the potential for elucidating the corresponding physiological processes, pathogenic pathways, or pharmacological responses. However, usually such biomarkers are found only at trace level concentrations within the human body.

Recent advances on the identification of disease biomarkers have attracted considerable scientific and clinical interest in the analysis of EB (vapor or condensate) providing a noninvasive diagnostic window for diagnostic purposes. It is evident that the composition of EB provides a complex image of the corresponding biochemical processes occurring within the body, which may be correlated to the physiological status of a patient. Hence, quantitative compositional analysis of breath may provide an attractive noninvasive diagnostic strategy for the recognition, diagnosis, and status monitoring of complex diseases and treatment strategies including but not limited to, e.g., breast

cancer, lung cancer, and pulmonary diseases. However, quantitative analysis of breath metabolites present at nanomolar to picomolar ($10^{-9} - 10^{-12}$ mol/L) concentration levels in real time remains a significant analytical challenge.

Nonetheless, it is evident that bodily secretions such as urine or EB contain at least subsets or breakdown products of biomarkers present within human body. In consequence, it is highly desirable to establish sensing schemes with sufficient sensitivity and molecular selectivity for addressing these constituents noninvasively at trace levels. Gas chromatography in combination with mass spectrometry (GC-MS) may be considered the current golden standard method for analyzing constituents relevant to breath diagnostics [3]. Despite excellent sensitivity, GC-MS usually requires preprocessing of breath samples and separation for addressing target analytes, which renders this method less suitable for analyzing samples in real time. While there are efforts in progress on miniaturizing GC and MS components, to date this technology remains for most applications confined to laboratory applications. In contrast, mid-infrared (MIR) spectroscopy has proven an excellent alternative methodology for trace level chemical detection with high sensitivity and molecular selectivity. Compared to GC-MS, MIR spectroscopy is appears more amenable to miniaturization and integration, therefore promising close to real-time analysis evaluating highly discriminative vibrational and rotational molecular signatures, which are critical merits for clinical applications on a routine basis.

The basic principle of the molecular level detection in the MIR at possibly compact device platforms utilizes a broadband light source or a tunable diode laser light source emitting radiation in resonance with fundamental vibrational and rotational modes associated with most inorganic and organic molecules that are spectroscopically accessible within the MIR (3–20 μm) regime of the electromagnetic spectrum. The interaction between MIR photons and particular organic molecules provides – compared to the near-infrared (NIR) and the UV/Vis range – comparatively sharp transitions. Despite the wide variety of organic molecules, the unique sequence of absorptions reflect the molecularly characteristic arrangement of chemical bonds within the probed molecules via the frequency position of the associated vibrational and mixed rotational-vibrational transitions. The spectral absorption patterns in the MIR are also considered molecular fingerprints, therefore rendering MIR absorption spectroscopy a powerful sensing technique for trace level chemical analysis.

II. Breath Analysis

The human body is considered among the most complicated living organisms in nature. To sustain its function, a complex metabolism progresses, which comprises a tremendous variety of biochemical reactions. During such metabolic reactions, a wealth of chemical/biochemical constituents are synthesized or transformed. Such physiological reactions are well balanced for within healthy human bodies, although there are variations depending on each individual metabolism or physiological status. As physiological reactions in response to diseases or treatment are different from healthy subjects, the status or progress of physiological and metabolitic processes should reflect in concentration changes of key constituents involved in or characteristic for specific reactions. While the majority of such biomarkers remain located inside the human body providing access only via invasive sampling of blood or biopsies, to a certain extent the pathophysiological condition should also be reflected in the composition of excreted bodily fluids such as urine, perspiration, saliva, etc., and breath as well as breath condensate. Hence, it is not surprising that some of constituents initially present with the human body may be determined either natively or after metabolitic transformation within such excretions. Each of these constituents may be considered a potential biomarker providing valuable information on the physiological status of the respective biological system, or the progression of a disease and/or therapeutic treatment thereof. However, given the complexity of such processes it has to be anticipated that rather than individual biomarkers – which do exist but are rarely sufficient for characterizing a physiological condition – entire panels of biomarkers will be up- and/or down-regulated during disease or treatment, yet above and beyond any naturally occurring patient-to-patient variation. Among these excretions, EB is considered a projection image – particularly of the volatile – chemical constituent profile of blood. While the alveolar membrane represents the interface between blood and air, where the exchange of O_2 and CO_2 takes place. However, any other molecular component permeating through this membrane barrier may finally be excreted via EB, therefore serving as potential biomarkers. In addition, EB contains nonvolatile compounds introduced via the lining fluid of the bronchial tract, the oral cavity, and/or the sinus. Among several advantages, diagnostics based on analyzing EB from patients has the distinct advantage of noninvasiveness. In particular, for patients in critical condition or neonates, complementing or replacing invasive methods such as, e.g., biopsies by EB analysis provides an attractive alternative. Furthermore, continuous access to naturally excreted samples enables (close to) real-time monitoring of the patient condition, which is difficult applying invasive testing and analyzing.

VOCs – and even less volatile constituents – are readily detectable in EB using appropriate analytical instrumentation usually in combination with advanced sampling techniques [3]–[7]. The matrix of EB is predominantly composed of N_2, CO_2, O_2, and H_2O along with minute amounts of trace constituents. The major constituents of EB reflect the inhaled breath composition with few exceptions. The concentrations of O_2 and CO_2 are different before and after breathing due to the alveolar gas exchange. Furthermore, EB includes trace amounts of organic and inorganic constituents permeating across the alveolar membrane from blood into the gas phase as a result of metabolitic or normal physiological processes, pathogenic processes, or a pharmacologic response. While compact diagnostics for EB analysis still remain in an early stage of research, there have been more than 1000 organic and inorganic constituents found in EB with concentrations ranging from the parts-per-million to the part-per-trillion regime by volume. While the comprehensive relationship between all the constituents in EB and the physiological processes or diseases is not yet completely understood, some diseases and the associated biomarkers detectable in breath are well studied. For illustration, Table I summarizes a selection of typical biomarkers and associated diseases.

TABLE I
SELECTED DISEASES AND BIOMARKERS FOUND IN EXHALED BREATH AND EXHALED BREATH CONDENSATE

Disease / Health status	Biomarkers in EB	Biomarkers in EBC	Reference
Breast Cancer	BMAC, pentane, formaldehyde		[37, 38]
Lung Cancer	BMAC, NO, acetaldehyde	H_2O_2, nitrite	[39, 40, 133]
Asthma	NO, CO	H_2O_2, leukotriene E4, isoprostanes, TBARs*	[27, 32, 134-138]
COPD	NO, CO	H_2O_2, leukotrienes, prostanoids, isoprostanes	[28, 33, 139, 140] [42, 141]
CF	NO, CO	H_2O_2, isoprostanes	[28, 34, 142]
ARDS	NO	H_2O_2	[143-145]
Diabetes	Acetone		[8, 12, 14, 16]
Schizophrenia	CS_2, pentane		[35, 36]
Renal Function	NH_3, dimethylamine, trimethylamine		[146, 147]
Liver function, Liver transplant rejection	COS**		[148, 149]
Helicobacter Pylori infection	^{13}C-urea breath test		[150-154]

*thiobarbituric acid-reactive products (TBARs); **carbonyl sulfide (COS)

A. Exhaled Breath (EB)

VOCs present in blood reflect in EB due to permeation across the alveolar membrane. The concentration of these VOCs will be affected by many factors including, e.g., the composition of inhaled breath, the concentration of VOCs in blood, and the partition coefficients of each individual component. The partition coefficient governs the relative concentration of each VOC as it distributes at the breath-blood interface. For a few VOCs, the partition coefficients at these conditions have been studied. Among others, typical partition coefficients are 2100:1 for ethanol, 330:1 for acetone, and $10 \sim 15 : 1$ for trichloroethylene [8]–[11].

The earliest and most extensively studied VOC in EB – with respect to the motivation of disease diagnostics – is acetone [8], [12]–[14]. Diabetes mellitus is a disease based on a metabolic disorder resulting in an increase of glucose concentrations and extensive lipolysis [15]. The liver produces ketones including acetoacetate, 3-β-hydroxybutyrate, and acetone, which are consumed as substitute energy resources. Consequently, diabetes induces increased blood ketones such that the concentration of acetone increases up to an equilibrium determined by the acetone partition coefficient across the alveolar membrane. Healthy individuals usually have acetone concentrations of a few hundred ppb in EB [14], [16]. In contrast, patients with diabetes reveal a considerable range of acetone concentrations in EB – as determined, e.g., by gas chromatography – depending on several parameters including the type of diabetes, age, sex, physiological status, etc.

As another example, the ^{13}C-urea breath tests (UBT) utilizing isotope labeling is a common method for detecting *Helicobacter pylori* infections in the stomach via the $^{13}CO_2/^{12}CO_2$ ratio. Such methods are suitable for detecting bacterial colonization and metabolism related to the gastroenterological system, pancreas, and liver [17]–[21]. *Helicobacter pylori* are known to cause gastric ulcers, duodenal ulcers, heart disease, and gastric cancers [22]–[24]. Orally administered ^{13}C-labeled urea is hydrolyzed by *Helicobacter pylori* in the stomach of the patient. This process produces ammonia and ^{13}C labeled CO_2, which diffuses into the blood matrix, and eventually shows up in EB where it can be used for *Heliobacter* infection diagnosis.

A variety of VOCs have been analyzed to diagnose respiratory infection and inflammation. Nitric oxide (NO) is a widely studied biomarker in EB, and serves as a nonspecific marker of inflammation in the lungs [5], [25], [26]. Also, NO is increased in atopic asthma and chronic obstructive pulmonary disease (COPD), but decreased in cystic fibrosis (CF) [27]–[30].

CO in EB was first studied as a potential biomarker in 1972 [31]. The major origins of CO in EB are categorized by exogenous processes, haem degradation, and nonhaem endogenous processes such as, e.g., lipid peroxidation or bacteria. CO in EB is also reported to increase in asthma, COPD, and CF [32]–[34].

CS_2 and pentane indicating peroxidation of polyunsaturated fatty acids were founded in EB from patients suffering from schizophrenia [35], [36]. However, the endogenous origin of CS_2, a potential specific biomarker for schizophrenia, is still unknown.

Last but not least, lung cancer appears detectable via different combinations of VOCs including, e.g., C4 to C20 alkanes, and monomethylated alkanes [37]. Below, we will discuss the relevance of VOCs in EB for breast cancer diagnosis [37]–[40].

B. Exhaled Breath Condensate (EBC)

Nonvolatile constituents in EB may also serve as potential biomarkers. While VOCs in EB mainly reflect the biochemical profile of blood, nonvolatile compounds predominantly in EBC are largely associated with biochemical changes of the surface lining fluids in the breathing airways starting from the alveoli to the nose and mouth. EBC is usually collected by a device with a cooled collection surface enabling condensation [5]. Depending on the collection conditions and the condenser temperature also some VOCs may be collected within EBC. In general, the composition of EBC has been less studied than, e.g., VOCs in EB. For example, elevated levels of H_2O_2, leukotrienes, lipid peroxidation products, and isoprostanes in EBC were reported from patients with asthma, COPD, or CF [41], [42]. These constituents are considered oxidative stress

biomarkers. Nitric oxide metabolites such as, e.g., S-nitrosothiols, nitrite, nitrate, and 3-nitrotyrosine in EBC have also been used as trace compounds of airway inflammation in asthma, CF , COPD, and acute respiratory distress syndrome (ARDS) [43]–[46]. Since the stability and reactivity of many reactive oxygen and nitrogen species are dependent on the pH, the actual pH of EBC is also an indicator of, e.g., reactive oxygen or nitrogen species. For asthma, a relationship between the pH of EBC and asthma severity was observed, while in CF patients a lower pH of EBC was associated with exacerbations [47]. Furthermore, adenosine – which is a metabolite of adenosine triphosphate degradation – elicits a broad range of effects via specific receptors. Hence, elevated EBC adenosine levels have been determined as useful indicators in patients with allergic rhinitis and asthma [48]–[50].

C. VOC Analysis for Noninvasive Breast Cancer Detection

Worldwide, breast cancer (BC) is both the most commonly diagnosed cancer among females and the most common cause of female cancer death. However, marked disparities in incidence, stage at diagnosis, tumor aggressiveness, treatment, and mortality exist at the global level [51]. Once considered a disease of affluent western nations, breast cancer incidence and mortality rates are rapidly increasing in most developing countries where detection and treatment options remain grossly inadequate [52]–[57]. Stage at diagnosis remains the most important BC predictive and prognostic factor, i.e., earlier stage detection results in more treatment options and better prognosis [58], [59]. Mammography screening remains the gold standard for early stage detection and resultant improved outcome, including decreased mortality. Presentation at very late disease stage is the major BC problem in most developing countries, where widespread use (or any use) of mammography is precluded by the lack of financial and technical resources. Furthermore, a higher risk for breast cancer and more aggressive tumors are observed in certain subpopulations [57], [60]–[68]. Finally, for women diagnosed with breast cancer, whether in technologically advanced areas or developing countries, there is an evident need for monitoring devices following treatment [57], [64], [69]. The lack of mammography in developing countries, the necessity for interval/more frequent screening for higher risk women or those at risk for more aggressive cancers at earlier ages, and the role of monitoring for reoccurrence in treated breast cancer patients demonstrate the crucial need for alternative screening methods in the United States and throughout the world.

The use of breath volatile organic constituent (BVOCs) analysis has a significant potential to meet these needs. The use of EB as a noninvasive cancer screening modality is receiving more and more attention, particularly for lung cancer. We have recently demonstrated the feasibility of using a simple sampling device (commercially available Markes Bio-VOC sampler combined with a rapid passive sampler) and gas chromatographic/mass spectrometric (GC/MS) analysis to differentiate BVOC patterns between healthy and breast cancer patients in a study of 20 healthy volunteers and 20 cancer patients [70]. During this study, we have substantiated the hypothesis that there is a link between the presence of breast cancer and the occurrence of certain BVOCs, and that a simple sample collection device and mass spectrometric analyses could adequately indicate the presence of this link. To investigate this relationship, the alveolar breath of each subject was collected by the subject breathing five times at five-minute intervals into the sampler containing a Radiello® passive sampler followed by analysis via thermal desorption/GC/MS (TD/GC/MS). Background air was collected passively with Radiello® samplers placed in the room during the breath sample collection time-period to measure potentially confounding atmospheric exposures by the subjects inhaling volatile organic compounds (VOC) during the sampling period. Each subject refrained from eating or drinking for 2 h prior to sample collection to minimize confounders as a result of recent food and beverage ingestion. A total of 383 BVOCs were identified in the breath in both subpopulations – control and cancer. Several of these compounds may originate from lipid peroxidation mechanisms, i.e., nonenal, hexenal, hexanal, methacrolein, and isoprene. These compounds were grouped according to chemical properties and then – using both identity and concentrations – used as the input into a nonlinear reduction procedure for classification analysis. We achieved an overall sensitivity of 72%, and a specificity of 64% resulting in a correct classification rate of approximately 77% for differentiating breast cancer cases from no cancer [70]. These percentages are derived from 10,000 simulations involving random partitions into training and validation subsets within the observed data. BVOC biomarker patterns were obtained by monitoring 383 BVOCs among all of the volunteers. The available data was randomly split into two subsets: training and validation. Each training sample consisted of 60% of case observations and 60% of control observations, thus leaving 40% of cases and 40% of controls for testing the performance of classifiers. This simulation task was repeated 10 000 times using different randomly selected training and validation sets. Performance results were averaged over all runs.

In conformance with other researchers, our results indicate that individual compound identification is not appropriate for determining the presence or absence of disease, but aggregate low-dimensional summaries and compound quantities result in specific patterns that can confirm disease. Our data show promising evidence that breast cancer patients can be differentiated from volunteers through distinct BVOCs. Furthermore, it clearly demonstrated that predictive screening may be possible with this innovative approach.

D. VOC Analysis for Exposure Monitoring

An area where breath analysis has a tremendous potential, though which has only been partially tapped is personal exposure monitoring [71]. Breath analysis for exposure monitoring has the advantage of being able to measure body burdens thereby providing data about the relationship between exposure and dose [72]. The TEAM studies demonstrated that exposure may not be closely related to emissions, thereby demonstrating the need for personal exposure monitoring and means to measure body burdens [72]–[75] found that for EB VOCs to be an effective and sensitive method to determine exposure body burdens. Their findings demonstrated that exposure VOC breath levels in known exposure level tests were predictable and reproducible across their volunteer population. Xu *et al.*

[76] successfully measured the dermal uptake of 1,1-dichloropropanone 1,1,1-trichloropropanone, and chloroform during showering by analyzing time concentration profiles via EB samples. The breath VOCs in 26 asthmatic Hispanic children living near major freeways and trucking routes were determined by Delfino *et al.* [77]. Eight VOCs were quantified in greater than 75% of the samples: benzene, methylene chloride, styrene, tetrachloroethylene, toluene, m, p-xylene, o-xylene, and p-chlorobenzene. Their results showed a weak association between exhaled benzene and asthma episodes.

VOCs exposure in occupational settings are important sources of adverse health impacts due to the potentially higher the ambient levels due to large-scale production, their use in manufacturing processes, and their high toxic potential. The biomonitoring of occupational VOCs exposures is important to identify dose-response effect before toxic effects are manifested. Breath analysis is one biomonitoring technique that has been applied to measure occupational VOCs exposures. Amorim *et al.* [78], [79] used the Bio-VOC sampler with solid-phase microextraction (SPME) for sample collection and concentration to measure benzene in breath of workers at gasoline stations and a control group. They detected an average benzene level of 8.2 ppb in the control subjects and 25.3 ppb in the gasoline station workers. Chen *et al.* [80] also used breath VOCs and personal exposures of gasoline station workers to determine the breath VOCs sources. They measured toluene, xylene and ethylbenzene the breath of 30 workers from 10 gasoline stations. The study of Chen *et al.* suggests that exhaled toluene and xylene levels are suitable for use as biological exposure indices even at the ppb-level of exposure.

E. Sampling Methods for EB Vapor

The majority of the current methods used for breath analysis have involved collection of the breath samples on an adsorbent media or in an evacuated canister followed by GC/MS analysis [81]. Pellizzari *et al.* [82] published a standard method for measuring VOC exposures via EB by having the subject breath into a Tedlar bag and then drawing the air onto a Tenax cartridge followed by GC/MS analysis. A spirometer mouthpiece was used to collect the alveolar exhaled air. Lin *et al.* [83] analyzed volatile carbonyl compounds in breath to investigate lipid peroxidation by collecting the breath samples on silica cartridges impregnated with 2,4-dintrophenylhydrazine (DNPH) with high pressure liquid chromatographic (HPLC) analysis. The subjects breathed into a Douglas bag from which the trapped breath was drawn onto the sampling cartridges.

The frequently used adsorbent media is one method of preconcentration used to date by most researchers of breath VOCs. The very low concentrations of many breath components (low ppb or lower range) necessitate enrichment to achieve detectable sensitivity for the analytical techniques [84], [85]. Organic polymers, activated charcoal, graphitized carbon, carbon molecular sieves, cryogenic trapping, and solid phase microextraction (SPME) are all methods that have been employed to preconcentrate a larger volume of sample to achieve detectable analytical sensitivities. Each of these methods apply technique biases and for the most part focus a large number of compounds somewhat equally rather than allowing the targeting of selected compounds of interest.

F. Direct Analysis of EB

Direct analysis of EB (vapor or condensate) is among the major challenges in breath diagnostics due to more than 1000 constituents found in EB. Their concentration ranges from the part-per-million (ppm) to the part-per-trillion (ppt) regime, which renders only few analytical techniques suitable to assess these compounds at trace levels including, e.g., gas chromatography (GC), mass spectrometry (MS), infrared spectroscopy, and chemiluminescence. GC – in part – in combination with MS – is probably the most common method to analyze breath relevant constituents at trace levels. L. Pauling has already used GC techniques to investigate human EB [2]. While GC can cover a wide variety of compounds with excellent sensitivity, it remains a rather sophisticated method usually bound to the laboratory, and of limited suitability for real-time analysis. Several different types of and combinations with MS also provide high sensitivity, but are likewise bulky and expensive to operate. Both techniques are highly suitable – and probably the gold standard – for laboratory studies, however, lack in practicability during everyday use in clinics or during field studies. Consequently, portable breath analyzers should be compact in size, enable – at least close to – real-time measurements, inherently trace multiple biomarkers, remain cost effective, and provide the demanded molecular selectivity and sensitivity at ppb to ppt (by volume) concentration levels. Hence, direct analysis methods of breath VOCs offer significant advantages. Complications of contamination from sampling media/systems, the loss of VOCs by adsorption onto the storage vessel, and/or collection biases resulting from the selection of the adsorbent media are reduced. Additionally, immediate results are attainable with direct analysis methods rather than waiting for the sample to returned to a laboratory for analytical analyses. Selected ion flow tube mass spectrometry (SIFT-MS) is one of the more commonly used direct analysis method for breath VOCs analysis [86]–[89]. Although SIFT-MS has been applied to breath analysis in real-time without the need for preconcentration, this instrument is a highly specialized type of mass spectrometer and is not readily available in many laboratories, particularly clinical laboratories; therefore, does not have wide applicability for population exposure studies. Proton transfer reaction mass spectrometry (PTR-MS) is another specialized mass spectrometer that allows the direct, real-time measurement of breath [90]–[92]. Once again this is an uncommon and expensive laboratory-based mass spectrometer that is not readily available in many laboratories; therefore, does not have a significant applicability for population exposure studies.

Electronic nose technology also has been applied to breath analysis; however, these generally have the drawback of nonselectivity [93]. Di Natale *et al.* [94] used an electronic nose consisting of eight quartz microbalances coated with different metalloporphyrins to investigate if breath VOCs could detect lung cancer by focusing on alkanes and aromatic compounds. Breath was collected from the subjects in a sampling bag from which the analysis was performed. Overall, using this analytical technique in conjunction with a biostatistical classification scheme was able to differentiate between cancer and noncancer in 94% of the cases.

In summary, the examples of noninvasive breast cancer detection discussed in $C.$, and exposure monitoring summarized

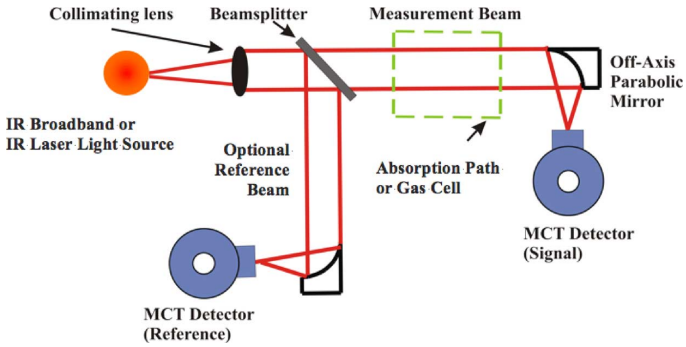

Fig. 1. Schematic of a measurement setup using an IR broadband (e.g., FT-IR) or IR narrowband (e.g., laser) light source for direct absorption gas sensing. IR radiation is selectively absorbed by analyte molecules within the absorption path defined by a single-pass gas cell, multipass gas cell, or hollow waveguide. A reference channel with a reference detector may be introduced, if a laser light source is used. (MCT— mercury-cadmium–telluride detector.)

in D. clearly indicate the need for portable sensing devices that are particularly capable of either direct or preconcentrated VOC detection. Therefore, the inherent molecular selectivity provided by MIR spectroscopic and sensing techniques promise an interesting suite of measurement techniques for advanced EB analysis.

III. Mid-Infrared (MIR) Sensing Techniques

MIR spectroscopy provides a sensing platform that appears ideally suited to fulfill the needs of clinically deployable breath analyzers with respect to the criteria listed above. In the following, this contribution reviews and compares various emerging MIR sensing techniques based on the types of MIR radiation sources and transduction principles with respect to their potential suitability for breath analyzer applications. Fig. 1 provides a general measurement scheme for IR absorption gas sensors.

A. Mid-Infrared (MIR) Light Sources

1) Sensing Based on Broadband MIR Light Sources: Fourier transform infrared (FT-IR) spectrometers combined with an appropriate gas cell establish a broadband MIR gas sensing system. SiC filaments used in FT-IR spectroscopy emit (black body) radiation covering the entire MIR range. In combination with hollow waveguide gas sensing modules, Mizaikoff and his research group have demonstrated sensitive detection of CO_2 and CH_4 with limits of detection (LOD) of 15.8 and 517 ppb utilizing such broadband sensing systems, i.e., a 1 m long hollow core waveguide (HWG), and a mercury cadmium telluride (MCT) detector [95]. The volume requires to fill the gas cell is only approx. 1.5 mL, which appears ideally suitable for, e.g., EB analysis. The general advantage of broadband MIR sensing systems is the wide spectral coverage so that most of the VOCs in EB can be assessed via their molecularly selective absorption signatures. However, the rather weak spectral energy density provided by this broadband emitter – in particular, in contrast to laser light sources – limits the achievable sensitivity. In addition, the obtained spectra frequently appear highly convoluted due to the anticipated complex molecular composition of the sample, which demands for sophisticated multivariate data evaluation and calibration techniques.

2) Sensing Based on MIR Laser Light Sources: In contrast to broadband emitters, MIR laser sources provide enhanced spectral density in a narrow emission band, which should facilitate target constituent analysis at trace concentration levels. While high spectral power density is a significant advantage for enhanced sensitivity, the lasing wavelength is usually fixed or may only be tuned across a narrow spectral range, thereby trading off some multiconstituent sensing capability. MIR laser sources include rather bulky gas lasers such as, e.g., CO_2 lasers ($9.2 \sim 10.8\ \mu\mathrm{m}$) and CO lasers ($5 \sim 6\ \mu\mathrm{m}$), devices for nonlinear optical wavelength conversion, and narrow bandgap semiconductor lasers and quantum cascade lasers (QCL). Though gas lasers provide the required MIR radiation for absorption spectroscopy, they have a few drawbacks such as complexity of operation, bulky dimensions and footprint, and limited tunability. Therefore, sensing systems based on gas lasers are predominantly limited to laboratory usage.

Wavelength conversion techniques generate MIR radiation by converting a fundamental pump beam of a visible or near-infrared (Vis/NIR) laser into radiation at MIR frequencies via nonlinear optical processes. Optical crystals with no structural inversion symmetry are able to generate such converted electromagnetic radiation (ω_1, idler) from a pump beam (ω_3, pump) with/without another input beam (ω_2, signal) taking advantage of the second-order nonlinear optical coefficient ($\chi^{(2)}$). This process is referred to as difference frequency generation (DFG) with ω_2 as external input, or optical parametric oscillation (OPO) without ω_2 as external input, respectively. The main advantage of frequency down conversion is the fact that mature Vis/NIR laser sources are readily available providing the required output power, beam quality, etc. Such arrangements may even convert Vis/NIR radiation into MIR radiation where compact or high-power coherent light sources were not available until the advent of QCL technology in 1994 [96]. For example, the research group of Tittel has developed compact laser different frequency spectrometers for multicomponent trace gas detection [97]. The sensing system based on this principle utilizes a periodically poled lithium niobate (PPLN) crystal as a nonlinear optical medium, which is pumped by a Nd:YAG laser (1064 nm, 700 mW), and a widely tunable external cavity diode laser ($840 \sim 865$ nm, 16 mW). Thus, an output frequency range from 3.98 μm to 4.62 μm may be covered. With this DFG spectrometer sensitivities down to 2 ppb for N_2O, 5 ppb for CO, and 100 ppb for CO_2 have been achieved [97].

Narrow Bandgap Semiconductor Lasers: While sensing systems equipped with gas lasers or conventional solid state lasers demonstrate excellent sensitivity owing to their high spectral power density, their bulky dimensions and limitations in tuning the emission range still limits their applicability, e.g., in clinical settings. Hence, it is essential to develop sensing systems based on more compact sized laser light sources enabling broad tenability of the MIR emission. The main development efforts for MIR laser sources have been focused on narrow bandgap semiconductor materials including, e.g., lead salt materials or IV-VI semiconductors, and were encouraged by the success of compound semiconductor lasers in the Vis and in

the NIR regime. These narrow bandgap semiconductor lasers operate by generating photons via radiative recombination of electrons from the conduction band, and holes from the valence band. Narrow bandgap lasers have been used as MIR sources within a wide variety of sensing studies with only few selected application examples illustrating their utility in breath-related applications here. For example, Roller *et al.* have utilized a IV-VI MIR laser to simultaneously determine exhaled NO and CO_2 in human breath for quantitatively discriminating between asthmatic and nonasthmatic exhalations [98]. In another study, Jeffers *et al.* have measured benzene in air with a detection limit of 1 ppm by volume, and an integration time of 4 s [99].

Narrow bandgap semiconductor lasers have several disadvantages, even though they represent a family of compact MIR laser sources. Most importantly, they usually require cryogenic temperatures (15–80 K) for operation at acceptable output power. Yet, their optical power remains low, i.e., in the range of several milliwatts and below. In addition, the achievable beam quality is limited and frequently inconsistent between laser diodes. Finally, they are limited in achievable emission frequency within the MIR spectral band, as the emission wavelength is largely governed by the bandgap provided by the involved material, which is difficult to engineer. In particular the need for cryogenic cooling adds to the complexity of a sensing system, and limits applicability in real-world scenarios. Furthermore, comparatively low optical output power as provided by narrow bandgap lasers limits the detection sensitivity of the sensing system to biomarkers present or changing their concentration at relatively high concentration levels (high ppb to ppm range).

Quantum Cascade Lasers: Most of the limitations associated with narrow bandgap semiconductor lasers are overcome by the latest generation of semicinductor laser light sources, namely quantum cascade lasers. QCLs emit photons by successive radiative intersubband transition of electrons within the conduction band without contribution from radiative recombination of electrons and holes. Resulting, intentional tailoring of the laser emission frequency is simply achieved by engineering the thickness of the layers composing the quantum heterostructure, rather than changing the composition. In addition to thus achieved flexibility in laser emission frequency selection, QCLs inherently provide significantly higher optical output power [100] exceeding 700 mW [101], whereas lead salt lasers usually emit only a fraction of this power (e.g., at 1.2 mW [102]) in multimode continuous wave operation [103]. Since the introduction of QCLs in 1994, numerous studies have demonstrated superior sensitivity and selectivity achievable utilizing this most advanced MIR light source for gas sensing applications in dependence of the type of gas cell that has been used, and the tunability of the QCL. For example, Charlton *et al.* have reported the detection of 30 ppb ethyl chloride within a 1.5 mL sample volume based on an integrated MIR sensing system utilizing a 1 meter long photonic bandgap fiber, and a QCL lasing at a wavelength of 10.3 μm [104], which expands previously reported results into the low ppb concentration range [105], [106].

Tunable Quantum Cascade Lasers: Application of a laser light source for biomarker detection in breath analysis has an advantage, if sensitive and molecularly selective detection can be achieved via frequency matching between the laser emission, and the absorption of a particular analyte. Most frequently, this match is not naturally achieved; therefore, the ability to precisely and deliberately tune the laser emission across a selected spectral range is of particular importance. Conceivably, an array of widely tunable QCLs could cover a range of hundreds of wavenumbers within the MIR band, therefore establishing a "miniaturized spectrometer" based on a series of QCLs, thereby quantitatively addressing a variety of different biomarkers within a breath sample. Initial methods to achieve QCL emission tuning have included adjusting the heat sink temperature using a cryostat, or using DC laser injection currents [107]–[109]; however, the spectral tuning range remained limited to a few wave numbers. Other approaches aim at tailoring the laser emission include the fabrication of an array of single-mode lasers monolithically fabricated on one chip with a variety of emission frequencies [108], or tailoring the cavity length of the QCL [110]. Perhaps the most effective method for widely tuning QCL emission frequencies has been the combination with or integration of external cavities, which are commonly known as external cavity quantum cascade lasers (EC-QCL). External cavity tunable QCLs (EC-QCLs) [111]–[115] operate by changing the angle of an external diffraction grating, which creates single mode emission via frequency selective feedback [100], thereby continuously tuning the laser emission across a spectral range determined by the properties of the grating; such devices may cover to date up to 250 cm^{-1} [113]. A recent study by Young *et al.*. presents selective and sensitive simultaneous detection of multiple analytes within a gaseous sample at ppb cocentration levels [116]. Using an EC-QCL combined with a hollow waveguide trace gas sensing module enabled precisely tuning the laser emission to selective absorption frequencies within a mixture of three gases, thereby simultaneously detecting these constituents at concentrations as low as 4 ppb for ethyl chloride, 7 ppm for dichloromethane, and 11 ppb for trichloromethane during exponential dilutionexperiments.

B. Transducers

1) Hollow Core Waveguides Versus Conventional Gas Cells: Quantitative direct absorption measurements rely on the Lambert–Beer law ($A = \varepsilon c l$ with $A = \mathrm{Absorption}$, $\varepsilon = \mathrm{molar\ Absorptioncoefficient}$, $c = \mathrm{concentration}$, and $l = \mathrm{optical\ path\ length}$). Hence, controlling the sensitivity of the device is largely based on selecting an appropriate optical path length, i.e., in most cases the length of the gas cell. As a result, the internal volume of a gas cell usually has to be large, such that the required optical path length for low detection limits may be achieved by folding the beam multiple times within the sample volume (Fig. 2). Such multipass gas cells offer enhanced sensitivity due to mirrors within the cell, which fold the IR beam for extending the optical path length up to several 100 m; however, the internal volume of such gas cells may reach 3 liters or more, which adversely affects the response time to concentration changes, and occasionally – e.g., in EB analysis – requires prohibitively large sample volumes [56]. Thus, gas cells with smaller internal volumes that still retain or improve the achievable sensitivity by ensuring intimate

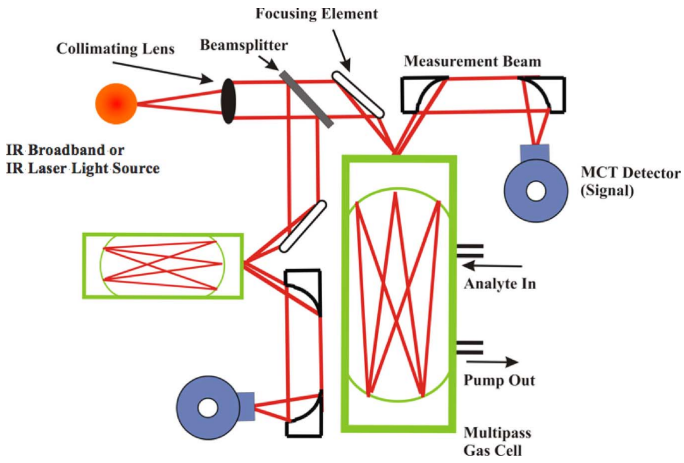

Fig. 2. Schematic of a measurement setup using an IR broadband (e.g., FT-IR) or IR narrowband (e.g., laser) light source for direct absorption gas sensing with a multipass gas cell. A reference channel with a reference detector is optional, if a laser light source is used. (MCT—mercury-cadmium–telluride detector.)

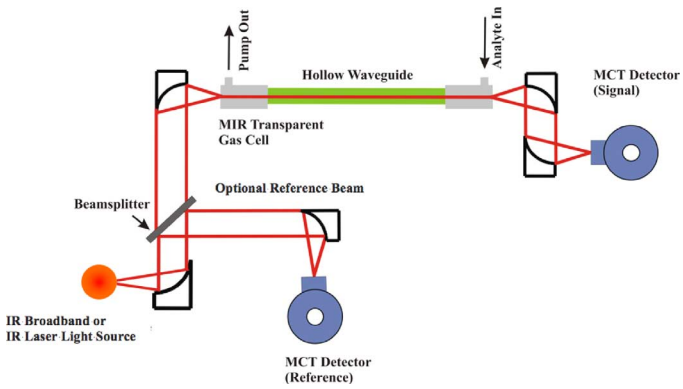

Fig. 3. Schematic of a measurement setup using an IR broadband (e.g., FT-IR) or IR narrowband (e.g., laser) light source for direct absorption gas sensing using a hollow waveguide. The hollow waveguide simultaneously acts as a gas cell and as a light pipe propagating IR radiation, thereby ensuring intimate contact between IR photons and analyte molecules within the hollow core. A reference channel with a reference detector is optional, if a laser light source is used. (MCT—mercury-cadmium–telluride detector.)

interaction between photons and molecules are required for taking full advantage of QCL-based chemical sensors for the detection of biomarkers in human breath.

A potential solution to these demands are hollow waveguide (HWGs) structures (Fig. 3). Such structures may be as simple as a structural silica tube, which is internally coated with a reflective layer of silver followed by a thin protective layer of silver iodide [117], or more sophisticated, a hollow core structure with cylindrical layers of, e.g., glass and polymer materials exhibiting alternating dielectric constants, which create a photonic bandgap [104], [108], [118] (termed photonic bandgap waveguides; PBG). QCL-based optical chemical sensors utilizing hollow waveguides have already demonstrated limits of detection down to a few ppb [104], [108] probing an internal volume (within the HWG or PBG) of only a few milliliters; such volumes appear advantageous for biomarker detection in breath. Furthermore, it is conceivable that coiled HWGs may be used for even further extending the achievable optical path length, although a balance between the benefits of the enhanced optical

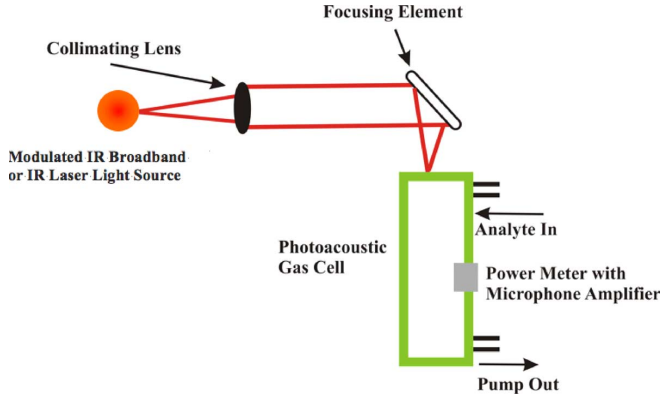

Fig. 4. Schematic of a measurement setup using a modulated IR broadband (e.g., FT-IR) or IR narrowband (e.g., laser) light source for photoacoustic gas sensing. Modulated IR radiation periodically heats the sample gas, thereby producing a pressure wave detectable at acoustic frequencies as a function of analyte concentration.

interaction path length, and radiation losses from bending the waveguide has to be determined [119].

2) Photoacoustic Transducers: Photoacoustic spectroscopy (PAS) [120] is another sensing technique that has been integrated with QCLs for trace gas sensing. PAS has the distinct advantage over other sensing technologies described herein that the signal transduction is not based on absorption, rather than pressure changes induced in a resonant or nonresonant sample compartment by modulated MIR radiation generating molecular vibrations (Fig. 4). The occurring fluctuations in pressure create a sound wave, whose intensity is a direct function of the gas concentration [100]. QCL-based PAS systems (QCL-PAS) conventionally use a device as simple as a microphone for transducing the sound wave, as documented during several studies with ultra-low sensitivity at ppb to parts-per-trillion (ppt) levels [121]–[124]. For example ammonia, which is considered a biomarker for asthma [5], was detected down to 66 ppb using QCL-PAS [122]. Most recently, a quartz tuning fork has been used in lieu of a microphone as the detector resulting in a technique known as quartz enhanced photoacoustic spectroscopy (QEPAS). Here, the resonance frequency of a quartz tuning fork is affected by the pressure fluctuations and resulting acoustic wave, if the probed molecules are present in between or close to the arms of the tuning fork. The major advantage of QEPAS with respect to the detection of biomarkers in breath is that the dimensions of the tuning fork are small (e.g., quartz tuning forks as used in conventional quartz wrist watches have been used for QEPAS), thus achieving ultra-low sample volumes down to $< 1\ \mathrm{mm}^3$. In turn, this small volume allows for an overall miniaturized sensor footprint (approx. $5\ \mathrm{mm}^3$ are typical dimensions), which is particularly important for handheld diagnostic applications [125].

3) Solid Core Waveguides: In solid core waveguides, MIR radiation interacts with analyte molecules via an evanescent field, which may be generated at the interface between waveguide and adjacent medium, if appropriate optical conditions are provided (i.e., a waveguide with higher refractive index than the surrounding medium). Thus, solid core waveguides or solid core optical fibers may serve as efficient optical transducers ensuring reproducible interaction between photons and molecules

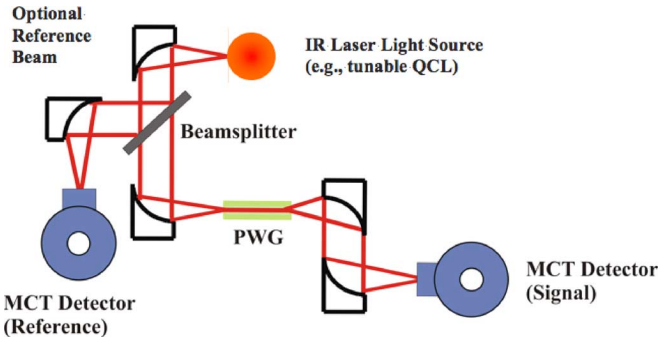

Fig. 5. Schematic of a measurement setup using an IR narrowband (e.g., laser) light source for liquid phase (e.g., breath condensate) evanescent field absorption sensing using a planar waveguide (PWG). A condensate sample is deposited onto the surface of the PWG, and analyte molecules present within the penetration depth of the evanescent field selectively absorb radiation at molecule-specific frequencies. A reference channel with a reference detector is optional, if a laser light source is used. (MCT—mercury-cadmium–telluride detector.)

at the waveguide surface, which may be modified in addition with analyte enrichment membranes or molecular recognition (bio)chemistry for attracting the probed molecules to the transducer surface. Of particular importance for MIR sensors is the surface modification with polymer membranes, which may not only enrich target analytes for detection with enhanced sensitivity, but may also selectively exclude a background matrix such as, e.g., liquid phase water from the analytically probed volume determined by the exponentially decaying evanescent field. Recently, Mizaikoff and collaborators have reported on the first liquid phase MIR sensing system comprising a QCL coupled with a thin film planar silver halide waveguide, and a QCL coupled to the first planar single mode MIR waveguide microfabricated from GaAs/AlGaAs, which is considered the first step towards fully integrated on-chip/on-wafer MIR photonic sensing systems [126], [127]. Based on such waveguides the detection of 80.7 μg of precipitated solid urea at the planar waveguide surface, and of 10.8 μg of acetic anhydride in solution (0.01 μL of acetonitrile) has been shown. Solid core planar waveguides are considered ideal candidates for EB condensate analysis, although their application is not limited to liquid phase or solid phase constituents (Fig. 5).

IV. Breath Diagnostics With MIR Sensors: Potential and Challenges

In the previous sections, the potential of biomarker panels found in EB (vapor and condensate) has exemplarily been highlighted, and the need for noninvasive diagnosis (e.g., in breast cancer screening) with particular reference to MIR spectroscopic and sensing techniques, which are capable of directly addressing relevant molecular signatures at trace levels, e.g., for VOCs has been discussed. Hence, detecting biomarkers in EB (vapor and condensate) using MIR sensing techniques possibly in combination with complementary sensing devices appears to provide an interesting strategy toward next-generation diagnostics in this emerging field.

A. Application of MIR Sensors in Breath Analysis

When considering the practical requirements for applying breath analyzers in the field, it is evident that MIR spectroscopic sensors are among the most promising technologies facilitating clinically deployable breath analyzers. In general, several of the MIR sensing systems discussed previously have initially been developed for the detection of trace level gaseous constituents in a wide variety of application scenarios. However, the exclusive molecular selectivity inherent to this frequency regime, the achievable detection limits, and the potential for miniaturization and integration suggests that this technology may significantly impact the field of breath diagnostics, which demands similar requirements.

Very recently, few academic and industrial studies have reported on translating IR technologies into devices suitable for breath analysis.

For example, Roller *et al.* have reported on the application of sensing systems utilizing tunable laser absorption spectroscopy (TLAS) to detect approximately 30 ppb of carbonyl sulfide (COS), which is considered a biomarker for failure of liver function, and for acute lung transplant rejection [128]. The TLAS sensing system comprised a thermoelectrically cooled MIR QCL lasing at 4.86 μm, and a 36 meter long optical multipass gas cell.

In another example, QCLs have been used as light source in integrated cavity output spectroscopy (ICOS) for detecting down to approx. 3.6 ppb NO during studies involving 15 exhaled human breath samples [129]. Ekips Technologies, Inc. has reported on the development of a tunable diode laser absorption spectroscopy (TDLAS) system utilizing IV–VI narrow bandgap semiconductor laser diodes emitting at 5.2 μm in combination with a 107 meter long multipass White cell for addressing exhaled NO and CO_2 [98]. The Ekips TDLAS sensing system was successful in discriminating asthmatic subjects from nonasthmatic subjects by the elevated level of exhaled NO. Furthermore, they have applied the TDLAS sensing system to studies in veterinary medicine during clinical trials of up to 800 subjects [130].

While clinical trials using MIR sensing techniques are yet rare, it appears that initial efforts and results are encouraging and should trigger further evaluation of this promising technology in breath diagnostics.

B. Challenges, Potentials, and Future of MIR Breath Analyzers

In the opinion of the authors, the main challenges for establishing clinically deployable MIR breath analyzers are twofold: i) development of MIR sensing systems reliably operating at ppb/ppt concentration levels and ii) identification of VOC biomarker panels traceable with MIR diagnostics.

Although the concentration of VOCs in EB (vapor and condensate) ranges from ppm to ppt levels, the sensitivity of the most practically applicable MIR sensing system remains at few tens of ppb. This sensitivity level currently limits access to biomarkers occurring at ppt concentrations, which would significantly broaden the spectrum of addressable VOCs in EB from currently only a handful of VOCs to an entire diagnostic panel. While device miniaturization is well on the way, the performance has to be enhanced for routinely addressing sub-ppb concentration levels. The advent of QCLs has tremendous impact on the utility and compactness of MIR sensing systems, and may be considered the single most important breakthrough for MIR technologies. However, of equal importance is the development of appropriate transduction schemes, i.e., waveguide

technologies, for ensuring efficient, reproducible, and quantitative interaction of photons with the molecular constituents. With the advent of the first semiconductor waveguides propagating MIR radiation for liquid, solid, and gas phase analysis a significant step forward toward compact sensing devices is evident. Eventually, handheld device dimensions pave the way toward personal health monitoring devices for direct *in situ* monitoring of the physiological status; clearly, IR technologies may be a vital component of such monitoring devices in combination with complementary sensing strategies. In addition, given the rather strong molecular absorptions of water vapor, MIR sensing schemes have to either minimize the impact of water vapor during the measurement by selecting appropriate spectral windows or tunable light sources, and/or take advantage of multivariate data evaluation schemes for deconvoluting the spectral contributions of water vapor and the constituents of interest. However, this is a general problem associated with IR spectroscopic measurement techniques, and has widely been addressed with a variety of strategies [131].

To date, breath analysis is still largely relying on single or few biomarker(s) detection, and the effort to attribute a certain condition to such few constituents, while human EB (vapor and condensate) is a complex matrix comprising more than 1000 VOCs. Hence, advanced EB diagnostics should take advantage of this wealth of information by addressing multiple constituents in parallel for accessing the physiological status of patients with more fidelity. This is of particular importance for complex disease pathologies, as encountered for various cancers detectable via breath, such as, e.g., lung cancer and breast cancer. Tracing the up- and down-regulation of individual constituents within entire biomarker panels is therefore a primary goal for next-generation diagnostic devices, however, requiring that fundamental research into, e.g., VOC panels characteristic for a certain diseases – as discussed for the case of breast cancer – remains a vital research area. In combination with state-of-the-art computational data analysis techniques and signal processing such massive data sets may be compressed to the characteristic variables relevant for certain disease pathologies, thereby defining the fundamental needs and demands for subsequently developed sensing technology.

In addition, continuous efforts into researching the medical relevance of VOCs is in demand, as only a few VOCs including, e.g., acetone or NO and their metabolitic importance are well understood, while the role of most VOCs remains ambiguous. For example, biomarkers such as CS_2 and their relationship with, e.g., schizophrenia are only in part understood lacking in depth understanding on the involved physiological processes. However, even if breath diagnostics come the proposed long way toward practical usage, precise interpretation of the obtained results ultimately relies on a thorough understanding of the underlying physiological importance.

Future efforts in developing next-generation breath diagnostics – and in particular – devices taking advantage of the inherent molecular selectivity associated with MIR sensing techniques – will have to overcome the diagnostic challenges described above including the technical challenges of reliable quantitative operation at the required levels of sensitivity, and ease of operation and handling during daily clinical usage. From an optimistic perspective, the expected simplicity of use and the anticipated comparatively low cost for a noninvasive diagnostic device allow anticipating an important role of such devices in preventive medicine, during widespread prescreening of patients, and during monitoring of therapeutic progress, in particular, in areas where more sophisticated or invasive diagnosis are not either not available, or cannot be performed.

More likely than replacing existing techniques, breath analysis via MIR sensing systems may evolve into a complimentary diagnostic method supporting currently existing diagnostic techniques including, e.g., biopsy, blood sampling, mammography, functional imaging, chromatographic separations, mass spectrometry, etc. In particular, MIR sensing systems may develop into an attractive option for less favored regions with limited access to sophisticated screening and analysis tool given its potential for miniaturization and ultimate cost effectiveness based on emerging integrated MIR photonics [132].

V. Conclusion

Though it has been a well-known fact that EB from patients may provide information on a disease or the health status for a long time, until recently EB has remained almost untouched by modern (analytical) chemistry or medicine. EB (vapor and condensate) contains more than 1000 chemical species ranging in concentration from a few hundred of ppm down to ppt levels. While VOCs in EB reflect the constituents also found in blood, nonvolatile compounds contained in EB condensate reflect biochemical changes related to the breathing airways within their lining fluid. Therefore, significant compositional and concentration changes of constituents within the EB (vapor and condensate) matrix may potentially serve as an indicator for diseases and/or the physiological status of an organism. A variety of constituents have already been established as identified biomarkers characteristic for certain diseases such as, e.g., acetone, alkanes, NO, CO, CS_2, COS, NH_3, H_2O_2, leukotrienes, nitride/nitrate, isoprostanes, etc. Not only pulmonary diseases, but also cancers, schizophrenia, diabetes, *Helicobacter pylori* bacteria infection, and others may be diagnosed by breath analysis. Among others, prevalent criteria for clinically deployable breath analyzers include sensitivity to ppt levels, sufficient molecular selectivity for discriminating individual biomarkers, a compact footprint, (close to) real-time monitoring, ease of operation, and cost efficiency. Evidently, MIR spectroscopy ranks among the most promising analytical techniques complementing currently applied and mostly laboratory-based analytical devices including, e.g., GC, MS, chemiluminescence, etc., in a more compact, ideally handheld format. In recent years, several MIR sensing techniques have evolved into analytical tools with sensitivities ranging from a few ppm to a few ppb yet with a rather compact device footprint. QCL technology has been a vital addition to the field of MIR sensing enabling compact breath analyzers with small yet bright coherent light sources tailorable to almost any wavelength across the entire MIR spectral band. Tunable EC-QCLs nowadays provide a tuning range of more than 250 cm^{-1}, thereby enabling the assessment of multiple biomarkers in complex mixture. With the introduction of semiconductor-based transducer/planar waveguide technology

ideally complementing QCL light sources, a next important building block for miniaturized sensing systems has recently been established. In addition, hollow core waveguides simultaneously serving as MIR waveguide and as a highly efficient gas cell are a promising approach for EB diagnostics, as a small sample volume ($\sim$ 1 mL) provides for an ideal transducer in MIR sensing systems compared to usually much larger conventional gas cells. PAS and QEPAS is another important transducer technology, which enables developing compact and sensitive sensing platforms in the MIR. For condensate (liquid) analysis, Charlton *et al.* were the first to demonstrate the utility of thin film planar waveguide technology coupled with QCLs for detecting liquid phase and solid phase analytes, which is an important first step toward MIR-based condensate analysis. Finally, first clinical trials using TLAS and TDLAS sensing systems demonstrate the emerging interest in bringing MIR technology to bear in a clinical setting, and certainly contribute to the acceptance of this measurement technique for breath analysis. While a more widespread application of MIR spectroscopy-based breath analyzers is desirable, a clinical implementation requires meeting the emerging challenges of improving the sensitivity of such devices along with a more comprehensive understanding of the relevant biomarker panels in EB and EBC.

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Charlene W. Bayer received the Ph.D. degree in organic chemistry from Emory University, Atlanta, GA, in 1981.

As a Principal Research Scientist and Head of the Environmental Exposures and Analysis Branch (EEAB) at the Georgia Tech Research Institute (GTRI), she has been responsible for numerous research projects in indoor environmental and exposure research. Prior to joining GTRI, she was a Research Chemist at Smith-Kline Corporation in antibiotic isolation and structure elucidation, Emory University, Department of Clinical Pharmacology, investigating drug metabolic byproducts, and the U.S. Geological Survey developing analytical methods for pollutant analyses in waters and sediments. Today her research has focused on mass spectrometric analyses of trace organic compounds in air. Her research interests include using breath volatiles analysis for breast cancer detection; studying the relationship between exposures and asthma exacerbation, development of real-time monitoring sensor systems for personal exposure monitoring, and investigating indoor environmental quality and its impact on the environment, human exposures, and learning and productivity in schools.

Boris Mizaikoff received the Ph.D. degree in analytical chemistry at the Vienna University of Technology, Vienna, Austria, in 1996.

Heading the Chemical Sensor Laboratory (CSL), he has been responsible for numerous research projects in the field of chemical IR sensors. In 1997, he was with the University of Texas, Austin, as a Postdoctoral Fellow. In October 2000, he finalized his Habilitation (Associate Professor of Analytical Chemistry) at the Vienna University of Technology. Since Fall 2000, he was a faculty member at the Georgia Institute of Technology, School of Chemistry and Biochemistry, heading the Applied Sensors Laboratory (ASL). Since 2004, he was Director of the Focused Ion Beam Center (FIB^2 Center) at Georgia Tech, and since 2005 a member of the Center for Cell and Molecular Signaling at Emory University, School of Physiology. In Fall 2007, he joined the faculty at the University of Ulm, Ulm, Germany, as a Chaired Professor heading the Institute of Analytical and Bioanalytical Chemistry. He is author/coauthor of over 130 peer-reviewed publications, 14 patents, and numerous invited contributions at scientific conferences. Today, his research interests focus on optical sensors, biosensors and biomimetic sensors operating in the mid-infrared spectral range, applications of novel IR light sources (e.g., quantum cascade lasers), system miniaturization and integration based on micro- and nanofabrication, multifunctional scanning nanoprobes (e.g., AFM-SECM), scanning probe tip integrated nano(bio)sensors, focused ion beam techniques, development of chemical recognition layers for separation and sensing applications (e.g., molecularly templated materials, functionalized sol-gels), chemometric data evaluation, advanced vibrational spectroscopic techniques (SEIRA, SERS), environmental analytical chemistry, process analytical chemistry, and biomedical diagnostics.